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| <p>(54) Title: USE OF COPPER SILICATE FOR THE CONTROL OF <i>LEGIONELLA</i></p> <p>(57) Abstract</p> <p>The use of copper silicate as a bactericide and more particularly for the control of <i>Legionella</i>, <i>Legionella</i> resistant soil compositions, potting mixes and air conditioning systems.</p>   |  |   |   |

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Use of Copper Silicate for the Control of *Legionella*FIELD OF THE INVENTION

The present invention relates to the use of copper silicate as a bactericide and more particularly for the control of bacteria of the *Legionella* genus, such as *L. pneumophila* and *L. longbeachae*. The present invention also relates to *Legionella* resistant potting mix, soil, compost and air conditioning systems.

BACKGROUND ART

Certain bacteria of the *Legionella* genus cause a pneumonia-like disease which can be fatal. Legionnaire's disease, which was first recognised in 1976, is one 10 such disease caused by *L. pneumophila*. Whilst *L. pneumophila* is difficult to cultivate in the laboratory it persists and is disseminated through infected water in air conditioning units. People become infected by breathing in vapour containing spores or bacteria.

*L. longbeachae* is another bacterial species that has caused a number of 15 fatalities. *L. longbeachae* can persist in compost environments including potting mixes and has caused the death of a number of people who have inhaled bacteria or spores from infected potting mix.

Copper silicate is a known pesticide and fungicide and in soluble or aqueous form is a particularly useful anti-snail and anti-slug agent. However, the 20 bactericidal properties of copper silicate have, until now, remained unreported. Moreover, the prior art is silent to the use of copper silicate as an anti-*Legionella* agent.

Whilst antibiotics may be used to treat *Legionella* infections, no economical and effective method exists to control *Legionella* in the environment. It is an object of 25 the present invention to provide a *Legionella* active composition and methods for

the control of *Legionella* in environments such as soils, composts and potting mix as well as air conditioning systems.

### DISCLOSURE OF THE INVENTION

The present invention provides a method for controlling bacteria of the *Legionella* genus, the method comprising the step of administering to said bacteria an effective amount of copper silicate.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers, but not the exclusion of any other integer or group of integers including method steps.

Throughout this specification, unless the context requires otherwise, the word "control", or variations such as "controls" or "controlling" will be understood to encompass the prevention of bacterial growth in environments where there are no bacteria, the inhibition of bacterial growth and the killing of bacteria.

- 15 The method of the present invention may be applied to control all *Legionella* species. However, the method is particularly useful for the control of *Legionella* selected from the group of species comprising *L. longbeachae*, *L. dumoffii*, *L. gormanii*, *L. micdadei* and *L. pneumophila* and even more preferably, *L. longbeachae* and *L. pneumophila*.
- 20 Preferably, the method of the present invention controls the bacteria by at least inhibiting their growth and even more preferably totally preventing their growth. In this regard, the copper silicate may act as a bacteriostatic agent. Alternatively, the copper silicate administered according to the method of the present invention may be lethal to the bacteria and thus act as a bactericide. Whether the copper silicate acts to inhibit growth or kills the bacteria depends at least partially on the amount of copper silicate administered and the medium in which the bacteria is located.

Thus, the present invention also provides a method for killing bacteria of the *Legionella* genus, the method comprising the step of administering to said bacteria an effective amount of copper silicate.

The copper silicate used in the method of the present invention is particularly

5 useful as a bactericide due to its ability to provide free copper ions in solution. This ability increases the activity of the copper silicate when compared to other copper compounds. When applied as a solution, the copper silicate adheres to surfaces and persists to extend its active life, thus providing additional advantages over other forms of copper.

10 The copper silicate may be applied in the method of the present invention in various forms and the particular form used is at least partially dependent upon the medium to which the copper is to be applied. For example, when the copper is applied to potting mix or some other solid or semi-solid medium, the copper silicate is preferably added as a solution. Alternatively, when the copper silicate

15 is to be added to a liquid or fluid medium it may be added as a solid or a solution.

When the copper silicate is applied in the method of the present invention as a solution it preferably comprises an aqueous solution of acidified copper silicate. The acidified copper silicate solution is especially preferred in the method of the present invention as it has a number of advantages when compared to other

20 copper compounds. These include: (i) persistent toxicity when compared to insoluble copper powders; and (ii) ease of application.

The method of the present invention may be applied to control *Legionella* bacteria wherever they may exist. Environments that may be particularly suitable for the application of the present invention are soils, compost and potting mix, as well as

25 air conditioning systems. These environments are known to support *Legionella* and have been the source of many cases of Legionnaire's disease.

Thus, the present invention also provides a method for controlling *Legionella* in a medium containing compost, such as potting mix or soil, the method comprising the step of administering to said medium an effective amount of copper silicate.

In the method immediately above, the *Legionella* is preferably *Legionella* 5 *longbeachae*, which is commonly found in compost environments including potting mix.

The present invention also provides a potting mix or soil composition including an effective amount of copper silicate, said effective amount being adapted to control *Legionella*.

- 10 Whilst it is yet to be confirmed, the copper silicate appears to act in at least one of the following ways. Firstly, the copper silicate may control the growth of amoeba in the compost environment thereby controlling the *Legionella* who may reproduce within the amoeba. Secondly, the copper silicate may act directly on the *Legionella*.
- 15 The present invention also provides a method for controlling *Legionella* in an air conditioning system, the method comprising the step of administering to said air conditioning system an effective amount of copper silicate.

In the method immediately above, the *Legionella* is preferably *Legionella pneumophila*, which is commonly found in the water used in air conditioning 20 systems.

The copper silicate may be applied to various parts of the air conditioning system as required. For example, the copper silicate may be applied as a coating, such as paint, to the inside surfaces of the tank which holds the reservoir water for the air conditioning system. Alternatively, the copper silicate may be applied directly 25 into the reservoir water.

As mentioned above, the effective amount of copper silicate may be varied depending on the particular application. When the copper silicate is in the form of an aqueous solution of acidified copper silicate, preferably SOCUSIL™ (trade mark of Sheen Biotechnology Pty Ltd and subject of US Patent 5,474,972), the 5 effective amount of copper silicate may be to a final concentration of at least approximately 0.0013% or at least approximately 0.0028%. In one particular form, the final concentration of copper silicate is approximately 0.0013% - 0.0056%.

When the copper silicate is applied to potting mix, the effective amount is to a 10 final concentration of at least approximately 0.011%, more preferably at least approximately 0.028%, even more preferably at least approximately 0.037% or still more preferably at least approximately 0.056%. In one particular form, the final concentration of copper silicate is approximately 0.011% - 0.056%.

The effective amounts of copper silicate for use in air conditioning systems may 15 be determined by one of ordinary skill in the art without undue experimentation using appropriate *in situ* and *in vitro* experiments. However, it is expected that the effective concentration of copper silicate in air conditioning systems will be comparable to those in soil environments.

The present invention will now be described with reference to examples. The 20 description of the examples is in no way to limit the generality of the preceding description.

## EXAMPLES

### General Materials

#### *L. longbeachae* strains

25 Several *L. longbeachae* strains were used in the examples. These included reference strains, clinical isolates, and environmental isolates. Table 1 lists all *L.*

*longbeachae* strains used along with their laboratory number, country of origin, and original source. All strains were obtained from the freeze dried culture collections at The Western Australian Centre for Pathology and Medical Research (PathCentre).

5 Table 1 : *L. longbeachae* strains used in the examples

| Organism              | Reference Number     | Country of Origin | Original Source      |
|-----------------------|----------------------|-------------------|----------------------|
| <i>L. longbeachae</i> | 92/L62<br>(364-H)    | Australia         | Potting Mix          |
| <i>L. longbeachae</i> | 93/L25<br>(353-Z)    | Australia         | Clinical isolate     |
| <i>L. longbeachae</i> | 97/I52<br>(7032133Y) | Australia         | Mulch                |
| <i>L. longbeachae</i> | 97/L54<br>(7032190N) | Australia         | Karri and peat mulch |
| <i>L. longbeachae</i> | 97/L55<br>(7032191P) | Australia         | Jungle mix           |
| <i>L. longbeachae</i> | ATCC 33462 Q44-1     | USA               | Reference Strain     |

Other *Legionella* species

Several *Legionella* species apart from *L. longbeachae* were used in the examples. These are listed in table 2 with their laboratory number, origin, and type. All strains were obtained from the freeze dried culture collections at the

10 PathCentre.

Table 2: Other *Legionella* species used in the examples

| Organism                             | Reference Number   | Country of Origin | Original Source  |
|--------------------------------------|--------------------|-------------------|------------------|
| <i>L. dumoffii</i>                   | 87/L11             | Australia         | Standard         |
| <i>L. gormanii</i>                   | 87/L14 (224-E)     | Australia         | Clinical isolate |
| <i>L. micdadei</i>                   | 87/L26 (238-H)     | Australia         | Clinical isolate |
| <i>L. pneumophila</i><br>Serogroup 1 | 98/L15 (18012926Y) | Australia         | Water Sample     |

Potting Mixes and Dirt

All potting mixes used are listed in table 3. All potting mix and dirt samples were stored at room temperature in sealed bags or containers. Fresh bags were used in the non-sterile potting mix experiments.

5 Table 3 : Potting mixes, dirt, and seed mix used in the examples

| Code | Type                                      |
|------|---|
| A    | Proprietary potting mix #1                |
| B    | Proprietary potting mix #2                |
| C    | Proprietary potting mix #3                |
| D    | Proprietary potting mix #4                |
| E    | Proprietary potting mix #5                |
| F    | Proprietary potting mix #5 + 0.5% SOCUSIL |
| G    | Proprietary potting mix #5 + 1% SOCUSIL   |
| H    | Proprietary seed mix                      |
| I    | Proprietary compost                       |
| J    | Proprietary compost + 0.5% SOCUSIL        |
| K    | Proprietary compost + 1% SOCUSIL          |
| L    | Dirt                                      |

Example 1: Testing of Copper Silicate Against *Legionella pneumophila***Reagents**

(1) *Legionella pneumophila* serogroup I (ATCC 33152)

(2) Copper silicate solution - 2.8g/L.

**5 Method**

(1) Prior to use, the copper silicate solution SOCUSIL was autoclaved to remove all existing organisms which were present. Autoclaving was carried out at 121°C and 103.5Kpa.

10 (2) 0.1mL of neat suspension of *Legionella pneumophila* serogroup I organisms (count of  $5.5 \times 10^6$  CFU/mL) was inoculated aseptically into 10mL of the autoclaved copper silicate solution. This gave a concentration of organisms in the copper solution of  $5.4 \times 10^4$  CFU/mL.

(3) The mixture was vortexed and placed in the incubator at 36°C.

15 (4) Samples of 50ul were removed at 1, 2, 4 and 24 hours and placed onto B.Y.C.E. media and horse blood agar.

(5) These subsamples were incubated at 36°C for 10 days.

(6) Examination of the culture were carried out every second day.

20 (7) Control cultures on B.Y.C.E media and horse blood agar were performed using 50ul of the suspension of the organisms without the presence of the copper solution.

## Results

Table 4

| Incubation            | B.Y.C.E Media  | Horse Blood Agar |
|-----------------------|--|------------------|
| 1 hr incubation 36°C  | No Growth  | No Growth        |
| 2 hr incubation 36°C  | No Growth  | No Growth        |
| 4hr incubation 36°C   | No Growth  | No Growth        |
| 24 hr incubation 36°C | No Growth  | No Growth        |
| Control Organism      | Group of <i>Legionella</i> (confirmed serologically) | No Growth        |

Example 2 - MIC of SOCUSIL (agar dilution method)

### 5 Methods

Preparation of media

To determine the MIC of SOCUSIL against *L. longbeachae* and relative to other copper compounds, BCYE agar was supplemented with various amounts of SOCUSIL, copper sulphate and malachite green. 20ml total volumes were used  
 10 and each solution was thoroughly mixed before pouring of plates. All plates were made in triplicate to ensure accuracy. Initially 2ml (1:10 dilution), 0.4ml (1:50 dilution), 0.2ml (1:100 dilution), and 0.1ml (1:200 dilution) of copper compound was added to BCYE agar. Based on these results, further dilutions were performed as required for each compound until the MIC was determined. Plates  
 15 were stored at 4°C for 24 to 48 hours before inoculation to reduce the risk of contamination.

Inoculation of plates

Individual suspensions of each of the bacterial strains listed in tables 1 and 2 were prepared in sterile distilled water and adjusted to the turbidity of a 1

McFarland turbidity standard on a Vitek colorimeter. 100 $\mu$ l of each suspension was added to 3 wells of a replica plate. Plates were inoculated by replica plating using a Denley machine resulting in an inoculum of approximately 10<sup>6</sup> bacteria/inoculum. Two BCYE plates were inoculated in the same manner to act 5 as controls. All plates were incubated in candle jars for 5 days at 36°C or until growth appeared on control plates.

## Results

The MIC of SOCUSIL is shown in table 5. The MIC for SOCUSIL of 0.0028% was the same for all *L. longbeachae* strains tested (refer to table 1 for complete 10 list) and was lower than the MIC for the other copper compounds tested. All bacterial strains grew on control plates after four days incubation.

Table 5 : Minimum inhibitory concentrations (MIC) using the agar dilution method.

| Chemical                  | <i>L. longbeachae</i> strains | <i>L. micdadei</i> | <i>L. dumoffii</i> | <i>L. pneumophila</i> Serogroup 1 | <i>L. gormanii</i> | <i>L. bozemanii</i> |
|---------------------------|-------------------------------|--------------------|--------------------|-----------------------------------|--------------------|---------------------|
| Copper Silicate (SOCUSIL) | 0.0028%                       | 0.0056%            | 0.0013%            | 0.0019%                           | 0.00056 %          | 0.028%              |
| Malachite Green           | 0.006%                        | n/t                | n/t                | n/t                               | n/t                | n/t                 |
| Copper Sulphate           | 0.014%                        | 0.014%             | 0.007%             | 0.007%                            | 0.007%             | >0.014%             |

nt - not tested

15 > = MIC is greater than the concentration tested

### Example 3 - MIC of SOCUSIL (in broth)

## Methods

To determine the MIC of SOCUSIL in broth, cultures were set up to contain the same concentration of bacteria but varying amounts of SOCUSIL as described in 20 table 6. *L. longbeachae* cultures were set up in sterile distilled water (SDW). A control containing only bacteria was set up for each to ensure the bacteria were alive, as was a purity control to ensure only *L. longbeachae* were present.

Table 6 : Preparation of cultures to determine MIC of SOCUSIL in broth

| Concentration of SOCUSIL | SOCUSIL (ml) | Bacteria (ml) | SDW/NB (ml) |
|--------------------------|--------------|---------------|-------------|
| 0.14%                    | 5            | 1             | 4           |
| 0.056%                   | 2            | 1             | 7           |
| 0.028%                   | 1            | 1             | 8           |
| 0.011%                   | 0.4          | 1             | 8.6         |
| 0.0056%                  | 0.2          | 1             | 8.8         |
| 0.003%                   | 0.13         | 1             | 8.87        |
| 0.0028%                  | 0.1          | 1             | 8.9         |
| Sterility control        | 5            | -             | 5           |
| Control (Survival)       | -            | 1             | 9           |

Viable counts were performed on each sample every 2 days for 6 days. Viable counts were performed by serially diluting 10 $\mu$ l amounts from each sample 10 5 fold until a dilution of 1:100000 was obtained. Three 10 $\mu$ l amounts from each dilution, including the culture itself, were spot plated and incubated on BCYE plates.

## Results

The MICs of SOCUSIL against *L. longbeachae* in broth are shown in table 7. 10 From the results, the MIC of SOCUSIL with a broth initially containing approximately  $2 \times 10^4$  *L. longbeachae*/ml was 0.056%.

Table 7 : MIC of SOCUSIL in broth against *L. longbeachae*

| Concentration of SOCUSIL | <i>L. longbeachae</i>        |
|--------------------------|------------------------------|
| 0.14%                    | -                            |
| 0.056%                   | -                            |
| 0.028%                   | $2.32 \times 10^3/\text{ml}$ |
| 0.011%                   | $6.34 \times 10^3/\text{ml}$ |
| 0.0056%                  | $1.09 \times 10^4/\text{ml}$ |
| 0.0034%                  | $1.90 \times 10^4/\text{ml}$ |
| 0.0028%                  | $2.03 \times 10^4/\text{ml}$ |
| Initial Concentration    | $2.15 \times 10^4/\text{ml}$ |
| <b>Sterility Control</b> | -                            |

- = No Growth

#### Example 4 - Effect of SOCUSIL on *Legionella* in Potting Mix and Dirt

## 5 Methods

### Preparation of *L. longbeachae*

*L. longbeachae* suspensions were prepared in sterile distilled water and adjusted to the turbidity of a 0.5 McFarland turbidity standard using a Vitek calorimeter. 5ml of these suspensions was added to 45ml of sterile distilled water and mixed 10 thoroughly. This 1:10 suspension, approximately  $10^6$  *L. longbeachae/ml*, was then used to seed potting mix and dirt samples.

### Preparation of potting mix and dirt

5g samples of potting mix A and dirt were weighed out into 50ml centrifuge tubes. All samples were then sterilised by autoclaving for 15 minutes at 121°C and used 15 that day.

Following sterilisation, sterile distilled water and the copper compound were added (to give a total volume of 2.5ml) to the soil samples to determine their effect in this environment. A starting concentration of four times their MIC in agar, as previously determined, was used, followed by higher amounts as 5 required. The samples were then seeded by the addition of 1ml of the *L. longbeachae* suspension. A sterility control was set up by the addition of 2.5ml of sterile distilled water to a sterile 5g potting mix or dirt sample. A control to ensure bacteria were surviving was set up by the addition of 1.5ml of sterile distilled water and 1ml of the *L. longbeachae* suspension to the soil samples. Four 10 samples of each chemical concentration were set up for each soil type to allow long term testing.

Following the addition of all substances, samples were mixed thoroughly by vortexing and then incubated for 1 and 7 days under aerobic conditions at 36°C.

Once the MIC was determined, potting mixes B, C, E, and H were tested in the 15 same manner but using a 0.056% and 0.08% concentration of SOCUSIL only. These samples were incubated overnight.

## Results

Results are shown in table 8. Initially only potting mix A and dirt were used to determine the concentration of SOCUSIL required to kill *Legionella* in their 20 natural environment.

The results obtained are shown in table 8 for potting mix A and table 9 for sterile dirt. After autoclaving, potting mix and dirt samples were sterile as indicated by a lack of growth of any organisms on BCYE and BA plates inoculated with the sterility control samples which contained only SDW.

25 The concentration required to kill approximately  $10^5$  *L. longbeachae*/5g of potting mix for SOCUSIL was 0.056% and for CuSO<sub>4</sub> was 0.56%. This value did not change after several days incubation. In sterile dirt, the amount required to kill

the same number of *L. longbeachae* was slightly less, being 0.028% for SOCUSIL and 0.14% for CuSO<sub>4</sub>, after seven days incubation.

Table 8 - Effect of SOCUSIL and copper sulphate on *L. longbeachae* in sterile potting mix A.

5 Day 1

| Compound & Concentration | No. <i>Legionella</i> /ml | No. in Original/ml  | Killed |
|--------------------------|---------------------------|---------------------|--------|
| 0.011% CuSi              | 1.15x10 <sup>6</sup>      | 1.7x10 <sup>6</sup> | 32%    |
| 0.028% CuSi              | 8.0x10 <sup>5</sup>       | 1.7x10 <sup>6</sup> | 52%    |
| 0.037% CuSi              | 1.07x10 <sup>4</sup>      | 1.8x10 <sup>5</sup> | 94%    |
| 0.056% CuSi              | No Growth                 | 1.8x10 <sup>5</sup> | 100%   |
| 0.084% CuSi              | No Growth                 | 1.8x10 <sup>5</sup> | 100%   |
|                          |                           |                     |        |
| 0.07% CuSO <sub>4</sub>  | 5.67x10 <sup>5</sup>      | 1.7x10 <sup>6</sup> | 66%    |
| 0.14% CuSO <sub>4</sub>  | 3.15x10 <sup>4</sup>      | 1.8x10 <sup>5</sup> | 83%    |
| 0.28% CuSO <sub>4</sub>  | 1.00x10 <sup>3</sup>      | 1.8x10 <sup>5</sup> | 99%    |
| 0.56% CuSO <sub>4</sub>  | No Growth                 | 1.8x10 <sup>5</sup> | 100%   |

Day 7

| Compound & Concentration | No. Legionella/ml    | No. in Original/ml   | Killed |
|--------------------------|----------------------|----------------------|--------|
| 0.011% CuSi              | 1.68x10 <sup>4</sup> | 1.73x10 <sup>5</sup> | 90%    |
| 0.028% CuSi              | 4.33x10 <sup>3</sup> | 1.73x10 <sup>5</sup> | 97%    |
| 0.037% CuSi              | 8.55x10 <sup>3</sup> | 1.35x10 <sup>5</sup> | 93%    |
| 0.056% CuSi              | No Growth            | 1.35x10 <sup>5</sup> | 100%   |
| 0.084% CuSi              | No Growth            | 1.35x10 <sup>5</sup> | 100%   |
|                          |                      |                      |        |
| 0.07% CuSO <sub>4</sub>  | 2.04x10 <sup>4</sup> | 1.73x10 <sup>5</sup> | 88%    |
| 0.14% CuSO <sub>4</sub>  | 1.05x10 <sup>4</sup> | 1.35x10 <sup>5</sup> | 92%    |
| 0.56% CuSO <sub>4</sub>  | No Growth            | 1.35x10 <sup>5</sup> | 100%   |

Table 9 : Effect of SOCUSIL and copper sulphate on *L. longbeachae* in sterile dirtDay 1

| Compound & Concentration | No. Legionella/ml    | No. in Original/ml   | Killed |
|--------------------------|----------------------|----------------------|--------|
| 0.011% CuSi              | 1.13x10 <sup>5</sup> | 1.87x10 <sup>6</sup> | 40%    |
| 0.028% CuSi              | 8.33x10 <sup>4</sup> | 1.87x10 <sup>6</sup> | 95%    |
| 0.037% CuSi              | No Growth            | 1.7x10 <sup>5</sup>  | 100%   |
| 0.056% CuSi              | No Growth            | 1.7x10 <sup>5</sup>  | 100%   |
| 0.084% CuSi              | No Growth            | 1.7x10 <sup>5</sup>  | 100%   |
|                          |                      |                      |        |
| 0.07% CuSO <sub>4</sub>  | 5.67x10 <sup>5</sup> | 1.87x10 <sup>6</sup> | 70%    |
| 0.14% CuSO <sub>4</sub>  | 2.33x10 <sup>5</sup> | 1.7x10 <sup>5</sup>  | 99%    |

Day 7

| Compound & Concentration | No. <i>Legionella</i> /ml | No. in<br>Original/ml | Killed |
|--------------------------|---------------------------|-----------------------|--------|
| 0.011% CuSi              | 4.33x10 <sup>5</sup>      | 1.17x10 <sup>6</sup>  | 63%    |
| 0.028% CuSi              | No Growth                 | 1.17x10 <sup>6</sup>  | 100%   |
| 0.037% CuSi              | No Growth                 | 9.04x10 <sup>4</sup>  | 100%   |
| 0.056% CuSi              | No Growth                 | 9.04x10 <sup>4</sup>  | 100%   |
| 0.084% CuSi              | No Growth                 | 9.04x10 <sup>4</sup>  | 100%   |
| 0.07% CuSO <sub>4</sub>  | 2.67x10 <sup>5</sup>      | 1.17x10 <sup>6</sup>  | 77%    |
| 0.14% CuSO <sub>4</sub>  | No Growth                 | 9.04x10 <sup>4</sup>  | 100%   |

Once the activity of SOCUSIL was determined, potting mixes A, B, C, E, and H were tested. The results are shown in table 10. At a concentration of 0.056% CuSi some *L. longbeachae* were recovered from potting mix A. In samples 5 containing 0.056% SOCUSIL, 99% of bacteria, which correlated to approximately 3.6x10<sup>5</sup> *L. longbeachae*/ml, were killed. No *L. longbeachae* were recovered from samples containing 0.084% CuSi.

Table 10 : Effect of SOCUSIL on *L. longbeachae* in other potting mixes

| Potting Mix | 0.056%<br>SOCUSIL        | % (& No./ml)<br>Killed       | 0.084%<br>SOCUSIL | % Killed |
|-------------|--------------------------|------------------------------|-------------------|----------|
| A           | 1.13x10 <sup>4</sup> /ml | 99.7 (3.64x10 <sup>5</sup> ) | -                 | 100      |
| B           | 3.67x10 <sup>3</sup> /ml | 99.9 (3.65x10 <sup>5</sup> ) | -                 | 100      |
| C           | 3.85x10 <sup>4</sup> /ml | 98.9 (3.61x10 <sup>5</sup> ) | -                 | 100      |
| E           | 3.60x10 <sup>3</sup> /ml | 99.9 (3.65x10 <sup>5</sup> ) | -                 | 100      |
| H           | 9.42x10 <sup>3</sup> /ml | 99.7 (3.64x10 <sup>5</sup> ) | -                 | 100      |
| Control     |                          | 3.65x10 <sup>6</sup> /ml     |                   |          |

10 - = No Growth

Example 5 - Effect of SOCUSIL on Plant Growth

**Methods**

To determine if SOCUSIL has any negative effects on plant growth, compost (brand I) and potting mix (brand E) samples were made up to contain either 0.5% or 1% copper silicate. 12 tomato seeds were then planted at the same depth and 5 spacing in the soil samples. The seedlings were then along with tomatoes planted at the same time and in the same manner but in normal seed mix. Growth of all seedlings was then monitored and plants measured and inspected to determine any effects on growth that SOCUSIL may have had compared to growth of plants in a SOCUSIL free environment.

**10 Results**

The average heights of the tomato plants are shown in table 11. Statistical analysis of this data is shown in table 12. SOCUSIL had no negative effects on plant growth at a concentration of 0.5% and 1%, and resulted in plants possessing thicker stems and larger leaves. In some cases, a significant 15 increase in average plant height was observed in seeds planted in soil containing SOCUSIL to that which did not.

Table 11 : Effect of SOCUSIL on plant growth

| Potting Mix | Average height of plants (cm) (SEM) |               |                |
|-------------|-------------------------------------|---------------|----------------|
|             | Day 0                               | Day 14        | Day 28         |
| I           | -                                   | 3.78 (0.4726) | 10.25 (0.5213) |
| J           | -                                   | 4.62 (0.6143) | 13.70 (0.7559) |
| K           | -                                   | 5.76 (0.3922) | 13.75 (0.7475) |
| E           | -                                   | 4.83 (0.6112) | 10.54 (0.7887) |
| F           | -                                   | 5.00 (0.6419) | 11.67 (0.7100) |
| G           | -                                   | 7.05 (0.4059) | 14.38 (0.5960) |
| H           | -                                   | 3.42 (0.2876) | 12.73 (1.0270) |

SEM = standard error of mean; E = Proprietary potting mix with no SOCUSIL; F = Proprietary potting mix with 0.5% SOCUSIL; G = Proprietary potting mix with 1% SOCUSIL; H = Seed Mix with no SOCUSIL; I = Compost with no SOCUSIL; J = Compost with 0.5% SOCUSIL; K =

5 Compost with 1% SOCUSIL; - = no growth

Table 12 : Statistical analysis of plant growth

| Potting mix results being compared | P value (significant difference) |            |
|------------------------------------|----------------------------------|------------|
|                                    | Day 14                           | Day 28     |
| E and F                            | 0.7639 (N)                       | 0.3006 (Y) |
| E and G                            | 0.0014 (Y)                       | 0.0009 (Y) |
| I and J                            | 0.2939 (N)                       | 0.0274 (Y) |
| I and K                            | 0.1302 (N)                       | 0.0009 (Y) |
| H and E                            | 0.0377 (Y)                       | 0.1052 (N) |
| H and F                            | 0.0284 (Y)                       | 0.4039 (N) |
| H and G                            | P<0.0001 (Y)                     | 0.0886 (N) |
| H and I                            | 0.2016 (N)                       | 0.0426 (Y) |
| H and J                            | 0.1762 (Y)                       | 0.7543 (N) |
| H and K                            | P<0.0001 (Y)                     | 0.4301 (N) |

E = Proprietary potting mix with no SOCUSIL; F = Proprietary potting mix with 0.5% SOCUSIL; G

= Proprietary potting mix with 1% SOCUSIL; H = Seed Mix with no SOCUSIL; I = Compost with no SOCUSIL; J = Compost with 0.5% SOCUSIL; K = Compost with 1% SOCUSIL; N = no

10 significant difference between the average height of plants in the two different soil types; Y = significant difference between the average height of plants in the two different soil types.

The present invention includes any and all modifications and adaptations apparent to one skilled in the art.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS

1. The use of copper silicate as a bactericide.
2. A method for controlling bacteria of the *Legionella* genus, the method comprising the step of administering to said bacteria an effective amount of 5 copper silicate.
3. A method according to claim 2 wherein the *Legionella* is selected from the group comprising: *L. longbeachae*, *L. dumoffii*, *L. gormanii*, *L. micdadei* and *L. pneumophila*.
4. A method according to claims 1 to 3 wherein the copper silicate is 10 administered as a solution.
5. A method according to claim 4 wherein the copper silicate solution is an aqueous solution of acidified copper silicate.
6. A method according to claim 4 or 5 wherein the copper silicate solution is SOCUSIL.
- 15 7. A method according to claim 2 or 3 wherein the copper silicate is administered as a solid.
8. A method for controlling *Legionella* in a medium containing compost, such as potting mix or soil, the method comprising the step of administering to said medium an effective amount of copper silicate.
- 20 9. A method according to claim 8 wherein the *Legionella* is *L. longbeachae*
10. A *Legionella* resistant potting mix or soil composition comprising an effective amount of copper silicate, said effective amount being adapted to control *Legionella*.

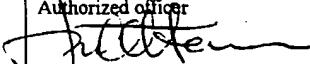
11. A method for controlling *Legionella* in an air conditioning system, the method comprising the step of administering to said air conditioning system an effective amount of copper silicate.
12. A method according to claim 11 wherein the *Legionella* is *L. pneumophila*.
- 5 13. A method according to claim 11 or 12 wherein the copper silicate is applied as a coating, such as paint, to the inside surfaces of at least a portion of the air conditioning system.
14. A method according to claim 11 or 12 wherein the copper silicate is applied directly into the reservoir water of the air conditioning system.
- 10 15. An air conditioning system comprising an effective amount of copper silicate said effective amount being adapted to control *Legionella*.
16. A method according to any one of the preceding claims wherein the effective amount of copper silicate is at least approximately 0.0013%.
17. A method according to any one of the preceding claims wherein the effective 15 amount of copper silicate is at least approximately 0.0028%.
18. A method according to any one of the preceding claims wherein the effective amount of copper silicate is at least approximately 0.0056%.
19. A method according to any one of the preceding claims wherein the effective amount of copper silicate is approximately 0.0013% - 0.0056%.
- 20 20. A method according to any one of the preceding claims wherein the effective amount of copper silicate at least approximately 0.011%,
21. A method according to any one of the preceding claims wherein the effective amount of copper silicate is approximately 0.028%,

- 21 -

22. A method according to any one of the preceding claims wherein the effective amount of copper silicate is at least approximately 0.037%
23. A method according to any one of the preceding claims wherein the effective amount of copper silicate is at least approximately 0.056%.
- 5 24. A method according to any one of the preceding claims wherein the effective amount of copper silicate is at least approximately 0.011% - 0.056%.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/AU 98/01008

|  |  |  |
|--|--|--|
| <b>A. CLASSIFICATION OF SUBJECT MATTER</b>   |  |  |
| Int Cl <sup>6</sup> : A61K 33/34   |  |  |
| According to International Patent Classification (IPC) or to both national classification and IPC  |  |  |
| <b>B. FIELDS SEARCHED</b>  |  |  |
| Minimum documentation searched (classification system followed by classification symbols)<br>IPC: A61K with electronic database search terms as below  |  |  |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched<br>AU: IPC as above  |  |  |
| Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)<br>WPAT : A61K 33/34IC and bacteri:<br>JAPIO : A61K 33/34IC and bacter:<br>CAS : copper silicate or Socusil and bacteri:<br>MEDLINE : copper or Socusil and bacteri: or legion:   |  |  |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>  |  |  |
| Category*  | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.  |
| X  | Derwent Abs Acc No: 97017174/02, Class C03 D22 E32 G02, JP 08-2283013 A (MIZUSAWA IND CHEM LTD), 29 October 1996   | 1  |
| Y  |  | 2-24   |
| Y  | Derwent Abs Acc No: 97431338/40, Class A97 D22 E37, JP 09-194313 A (ENDO, H) 29 July 1997  | 1-24   |
| Y  | Derwent Abs Acc No: 94140926/17, Class A60 D22 E32 F06, JP 06-087713 A (SANGI KK) 29 March 1994  | 1-24   |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C  |  | <input checked="" type="checkbox"/> See patent family annex  |
| * Special categories of cited documents:<br>"A" document defining the general state of the art which is not considered to be of particular relevance<br>"E" earlier application or patent but published on or after the international filing date<br>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)<br>"O" document referring to an oral disclosure, use, exhibition or other means<br>"P" document published prior to the international filing date but later than the priority date claimed |  | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention<br>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone<br>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art<br>"&" document member of the same patent family |
| Date of the actual completion of the international search<br>19 January 1999   | Date of mailing of the international search report<br>-5 FEB 1999  |  |
| Name and mailing address of the ISA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200<br>WODEN ACT 2606<br>AUSTRALIA<br>Facsimile No.: (02) 6285 3929   | Authorized officer<br><br>J.G. HANSON<br>Telephone No.: (02) 6283 2262 |  |

**INTERNATIONAL SEARCH REPORT**

International application No.  
PCT/AU 98/01008

| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |  |                       |
|---|--|-----------------------|
| Category*   | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
| Y   | Derwent Abs Acc No: 93-080295/10, Class A60, D22, E32, JP 05-025012 A (HAGIWARA GIKEN KK) 2 February 1993  | 1-24                  |
| Y   | Derwent Abs Acc No: 80253 E/38, Class C03, JP 05-7131708 (RIKAGAKU KENKYUSHO (TOMO)), 14 August 1982   | 1-24                  |
| X   | Patent Abstracts of Japan, C-858 page 76, JP 03-120221 A (MOZUSAWA IND CHEM LTD) 22 May 1991   | 1                     |
| Y   | Patent Abstracts of Japan, C-640 page 54 JP 1-167212 (SHINAGAWA NENRYO KK) 30 June 1989  | 2-24<br>1-24          |
| Y   | AU 27621/92-A (SHEEN AND LANGLEY) 22 July 1993   | 1-24                  |
| Y   | AU 25079/92-A (USA, DEPARTMENT OF HEALTH AND HUMAN SERVICES) 9 June 1994   | 1-24                  |
| A   | Seminars Resp Infects, Volume 13 No: 2 (1988) pages 147-159, Yu-sen et al, "Disinfection of Water Distribution Systems for Legionella"   |                       |
| A   | App and Envir Microl Volume 60, No: 5 (1994) pages 1585-1592, Rogers et al "Influence of Temperature and Plumbing Material Selection on Biofilm Formation and Growth of Legionella Pneumophilia in a Model Portable Water System Containing Complex Microbial Flora" |                       |

## INTERNATIONAL SEARCH REPORT

## Information on patent family members

International application No.  
**PCT/AU 98/01008**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in Search Report |          |      |          | Patent Family Member |       |    |         |
|--|----------|------|----------|----------------------|-------|----|---------|
| AU                                     | 27621/92 | EP   | 6092285  | SG                   | 47493 | US | 5474972 |
|  |          | WO   | 93/07754 |                      |       |    |         |
| AU                                     | 25079/92 | NONE |          |                      |       |    |         |